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DISINFECTION OF MARKETABLE EGGS BY PLASMA-CHEMICALLY ACTIVATED AQUEOUS SOLUTIONS

O. Pivovarov, Doctor of Technical Science, Professor
O. Kovalova, Candidate of Technical Science, Assistant Professor
V. Koshulko, Candidate of Technical Science, Assistant Professor
Department of the technology of storage and processing of agricultural products
Dnipro State Agrarian and Economic University
25, Serhii Yefremov str., Dnipro, Ukraine, 49600

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Correspondence:

O. Kovalova
E-mail: livre@i.ua

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Abstract. Microbiological purity of raw materials and finished products is one of the most important factors of food safety. The acceptable level of microbiological contamination is provided by a set of sanitary and hygienic techniques in the course of manufacturing, selling, and consuming food products. It has been suggested that eggs should be disinfected by treating them with plasma-chemically activated aqueous solutions in order to reduce microbial contamination of the egg surfaces and remove salmonella from them. The surface of eggs is disinfected with aqueous solutions activated by non-equilibrium contact plasma with the different time of activation of these solutions. Plasma-chemically activated aqueous solutions act as high-quality disinfectants. They contain an active substance (hydrogen peroxide) with the concentration of 100 to 700 mg/l, and have pronounced antiseptic properties. The studies have revealed that with the use of plasma-chemically activated aqueous solutions as a disinfectant, the number of bacteria and fungi on the eggshell is significantly reduced. For example, *E. coli* and *S. Enteritidis* were not found in samples at the peroxide concentration as high as 100 mg/l, while the total bacterial contamination and pathogenic microflora under study (*E. coli*, *Asp. fumigatus*, *S. Enteritidis*) were absent at 300 mg/l concentration of peroxides. Besides, our research included bacteriological studies of marketable eggs in storage. Thus, plasma-chemically activated aqueous solutions with the peroxide concentration 300 mg/l, besides destroying the pathogenic microflora completely (as evidenced by the total absence of QMAFAnM, CFU/cm^3, *S. enteritidis, E. coli, Asp. fumigatus* in the studied samples), had a long-term disinfection effect: no pathogenic microflora appeared on the surface of the eggs within a month. This will allow extending the shelf life of marketable eggs. The studies of microbiological parameters of the dirty eggshells treated with plasma-chemically activated aqueous solutions have shown that the 400 mg/l concentration of peroxides allows getting rid of coliform bacteria, staphylococci, salmonella, spore-forming microflora, which makes a product saleable. The outward appearance of raw materials improves as well, which indicates a wide range of applications of the proposed technology of egg surface disinfection.

Keywords: plasma-chemical activation, aqueous solutions, disinfectant, hydrogen peroxide, chicken eggs, contamination.

Introduction. Formulation of the problem

Food products should be safe for consumers. Microbiological contamination is one of the most serious risk factors, and the demands to its level include controlling both the total microbiological contamination and the presence/absence of certain types of particularly dangerous pathogenic microorganisms. The acceptable level of microbiological contamination is provided by a set of sanitary and hygienic conditions of preparation and packaging of food products.

Heat treatment is the most effective way to reduce the microbiological contamination of food products. However, it leads to irreversible changes in the properties of raw materials, which is unacceptable for marketable eggs. The use of chemical methods involves a large number of preservatives. That is why to increase the shelf life of food products, heat treatment is applied followed by cooling to the temperatures at which the reproduction of microorganisms stops.

Thorough and safe disinfection of food raw materials and products is a matter of particular importance. In today's context, epidemiological safety is still an acute problem. There are more than a thousand disinfectants currently available on the market. However, not all of them meet standards of reliability, efficiency, and safety. The reason is the selective action of such agents on the pathogenic microflora. This does not allow choosing an all-purpose germicide, which would effectively disinfect a product. Besides, most agents are quite toxic. So, the search for "green" chemical disinfection agents is still underway, and their development is of great interest to food producers.

Analysis of recent research and publications

A chicken egg contains the shell (12%), egg white (56%), and egg yolk (32%) [1]. Fresh eggs obtained from healthy birds do not contain any microbes. An egg remains sterile for a long time, since it has immunity. Bactericidal properties of the shell and egg white play a significant role in the immunity. The shell protects the egg from the penetration of microorganisms. Contamination occurs through the pores, the number of which reaches about 100 per 1 cm². Today, several forms of egg contamination in an industrial environment are known. These are: endogenous (microorganisms enter an egg during its formation in the ovary or oviduct of sick birds, who are often latent carriers of infectious disease agents and lay eggs containing viruses, bacteria, mould fungi, pathogens of salmonellosis and tuberculosis); exogenous (contamination of the shell with manure, litter, soil, feathers, etc.); contamination during collection, storage, and transportation. The presence of contaminants on the shell surface is the main cause of food poisoning [2-4].

According to data of the Centres for Disease Control and Prevention (CDC) [5], the company Rose Acre Farms in Seymour, Indiana, USA, voluntarily withdrew 206,749,248 eggs, because, according to preliminary analysis, they were infected with *Salmonella Braenderup* causing serious and sometimes fatal infections in young children, elderly people, or people with the weak immune system.

Healthy people infected with *Salmonella Braenderup* can experience fever, diarrhoea, nausea, vomiting, and abdominal pain. In rare cases, *Salmonella Braenderup* enters the bloodstream and causes more serious diseases such as arterial infections (for example, infectious aneurysms), endocarditis, and arthritis. After analysis of CDC data in the relevant reports, we see a steady trend to detection of outbreaks of diseases caused by pathogenic bacteria contained in eggs in different states for almost 40 years. Every time, the experience led to interdisciplinary training among students on investigating a foodborne disease outbreak, which is very important to prevent such outbreaks [6]. There were similar reported cases of food poisoning caused by infected eggs in Austria, France, and Germany. In these cases, the eggs were distributed by the same egg packaging centre in Germany, according to the data of the European Centre for Disease Prevention and Control/European Food Safety Authority (ECDC / EFSA) [7]. Bacterial contamination was also found on the surface of eggs collected in Korea [8].

Contamination of eggs increases due to the insanitary conditions of nests, of containers for egg storage, and of packaging material. The rate of microbe penetration into an egg is influenced by the temperature, high humidity, degree of freshness of eggs, presence of motility organs in bacteria, and other factors.

A wet eggshell is the most permeable to microorganisms. Conditions especially favourable for penetration of microorganisms into an egg are fluctuations in the air temperature, when the air containing pathogenic microorganisms is sucked in through numerous pores. At the temperature 20°C and relative humidity 80–85%, the bacteria *Pseudomonas* and *Proteus* penetrate from the shell surface into the egg on the 2^{nd}–5^{th} day, *Salmonella typhimurium* on the 8–11^{th} day, *E. coli* on the 13–15^{th} day, and *Aspergillus* on the 5–9^{th} day. Penetration of mesophilic microbes at the temperature 15°C and humidity 60–65% slows down, and below 10°C, almost stops. Psychrophilic bacteria of the genus *Pseudomonas* and mould fungi pass through the pores in a shell even at 0 [9].

Contaminated shell worsens the outer appearance of an egg and dramatically reduces the period of storage. Depending on the shell contamination, the number of microorganisms on it varies widely. There are tens and hundreds of bacteria on 1 cm² of fresh clean eggs, and tens of thousands and even millions of microbial cells on contaminated fresh eggs. Contamination of eggshells with pathogenic and potentially pathogenic microflora occurs in the systems of floor housing of chickens in poultry houses with poorly equipped nests, low-quality litter, and improper microclimate.

Eggs contaminated with pathogenic microorganisms can cause diseases in people who consume them. Salmonella, on getting into eggs, freely develops in them, since lysozyme (protein with antiseptic properties) has no effect on it. *S. enteritidis*, *S. choleraesuis*, *S. typhimurium*, *S. newport*, *S. dublin*, *S. anatum*, etc. [10] are the most dangerous salmonellae.

Besides salmonella, *Vibrio cholerae* and other pathogenic microorganisms, including causative agents of tuberculosis, can penetrate an egg through pores of the shell. *M. avium* [11] is most often found in chicken eggs. Eggs obtained from hens ill with tuberculosis or suspected of this disease are only used for the food purposes at food industry enterprises after heat pre-treatment. Selling of such eggs through retail chains or public catering enterprises is prohibited.

Eggs are disinfected by immersing them in a bath with a process solution of an agent, and the time of disinfection depends on the concentration of the solution. At the end of the disinfection time, the eggs are washed with running water and placed for drying. In modern poultry farms, edible eggs are virtually unaffected by harmful factors, since eggs laid by a laying bird move on the conveyor belt to the sorting shop, where they are divided into categories and packed in trays. Marketable eggs are disinfected before packing into boxes in order to prevent their colonisation by pathogenic microorganisms. At some poultry farms, eggs are processed, first in the sorting shop and then before packing.

Marketable eggs are disinfected after sorting and candling on an egg tester. Formaldehyde or acid disinfectants based on peracetic acid are used as disinfecting solutions. Formaldehyde is a toxic substance having a negative impact on the environment, so formaldehyde disinfection is becoming a thing of the past, being replaced by more effective and safer acid-based solutions [11,12].

Washing improves the appearance of eggs, but significantly reduces their stability during storage, so it is used immediately before breaking the eggs. Washing can also lead to the opening of pores in the shell through which microorganisms penetrate, and is accompanied by consumption of hot water (of about 80°C) and chemical disinfectants (2–3% of hydrogen peroxide, 0.5% of chloramine solution, and others), thus significantly increasing the environmental pollution. Sanitisers with a detergent effect, allowed in the food industry, are used too [13].

In the USA, this problem was solved simply: since the mid-1970s, eggs at poultry farms are washed and subjected to special disinfection treatment. However, it can actually have the opposite effect. According to recent research, the methods of treating eggs at American poultry farms destroy the special protective layer on the shell, which is a natural barrier to various infections. As a result, American eggs always look clean and good (an American eats about 250 chicken eggs a year, and 20% of deaths from cardiovascular diseases in the USA are due to egg yolks high in cholesterol, while in Japan consuming annually 329 eggs per capita, this figure is only 11%) [10], but they are much less protected than ours or European eggs. There is also a correlation between egg consumption and the risk of stroke in humans [14].

Hydrogen peroxide and agents based on it have a wide range of antimicrobial effects, for example, on coliform bacteria, staphylococci, streptococci, moulds, etc. It can be used to disinfect any raw materials. Hydrogen peroxide mixed with other substances is used to sterilise surfaces. Hydrogen peroxide-based disinfectants are widely used in the food industry for disinfection. However, hydrogen peroxide and agents based on it are rather expensive, so the cost of products increases significantly [15,16].

Therefore, the search for high-quality harmless antiseptic agents continues. Particular attention is paid to the electrochemical treatment of water and aqueous solutions in order to change the chemical composition of a solution. A promising industrial method is the treatment of water with low-temperature (“cold”) non-equilibrium contact plasma (NCP) and its application in the technology of decontaminating marketable eggs.

The processes occurring in a liquid medium in contact with NCP are of interest from the point of view of studying the reactions under non-equilibrium conditions, involving electrons and excited particles, the average energy of which is slightly higher, compared to that in the “cold” state. Chemical transformations during the contact action of a plasma discharge on the liquid take place mainly at the gas-liquid interface. Therefore, it is important to determine the parameters of plasma, the surface and volume changes in the gaseous and liquid media, and the mechanism of chemical reactions in the reaction volume [17].

Objects of research are chemically pure water and aqueous solutions of inorganic and organic composition. This has allowed us to get a holistic view of the processes in liquid media during their treatment with plasma discharge. The NCP is implemented at the junction of high-energy chemistry and classical electrochemistry, with the chemical and physicochemical processes involving high-velocity, excited, or ionised particles, the energy of which often exceeds that of chemical bonds. This energy creates specific conditions in the reacting system, and they, in turn, cause the emergence of new types of processes compared with the traditional ones [17].

Processes under consideration can be conventionally classified into electrochemical processes (involving transformations on electrodes) [18,19] and plasma-chemical ones (when ultraviolet radiation and ionisation of a gaseous medium with the formation of charged particles produce contact action on a liquid-phase system) [20].

The main role in the description of these processes is played by non-equilibrium chemical kinetics, which is characterised by the violation or absence of some or all typical features of classical kinetics. Free electrons take a significant part in plasma-chemical reactions: in most cases, reactions under their impact are decisive in the initiation of complex multistage chemical processes [21].

Their collisions with the particles of the plasma-forming gas lead to ionisation (formation of an electron and a positive ion). A condition for the stationary existence of plasma is the equal rates with which charged particles form and die. The energy of ionisation of a molecule exceeds the excitation energy of any of its internal degrees of freedom, that is why rotationally, vibrationally, and electronically excited states of molecules (including the radiant ones) form and decompose (dissociate) in plasma simultaneously. The particles resulting from collision under the action of an electron impact can react both with each other and with the materials that are in contact with plasma.

Various physical fields, such as electromagnetic, electric, magnetic, and hydrodynamic fields, are of great importance too [21].

A chemical reaction is one of the channels of energy redistribution in a system, which in turn brings it to a state with minimal potential energy. Continuously gaining energy, electrons transfer it to atoms and molecules by collisions. However, because of the relatively low efficiency of this transfer, there is a large difference between the translational energy of electrons and that of heavy particles. The function of electron energy distribution is non-Maxwellian, i.e. it cannot be characterised, for example, by the temperature parameter. It begins to depend on the composition of the gaseous phase and on the electric field strength. Specific energy density in plasma is so high that events in it cover a complex set of physical, physicochemical, and chemical processes [22], and the typical durations of all processes are close values.

So, they influence each other. That is why describing plasma-chemical reactions and processes occurring during their interaction is a fundamental multi-channel problem, with channels interacting differently depending on the various periods of time and energies. When we convert the average energy of electrons into the corresponding thermal units, the typical values of electron "temperature" will be $3.0 \cdot 10^5$–$1.0 \cdot 10^6$ K. Non-equilibrium phase transitions can be observed in highly non-equilibrium plasma (average energy of electrons ~ 3–5 eV, temperature of heavy particles ~ 300–500°K). For non-equilibrium conditions, it is possible to direct selectively the energy flow to activate the required components of a chemically reacting system.

Water and products of its dissociation, hydrogen and hydroxyl ions are the important factors, which determine the structure and biological properties of living organisms [23].

The strength of bonds between water molecules is determined by their electrical polarity and explains the specific arrangement of electrons in the oxygen and hydrogen atoms constituting the water molecule. The oxygen atom combines the pair of its electrons with the electrons of the hydrogen atoms by overlapping the *1s*-orbitals of the hydrogen atoms with hybrids of the sp^3-orbitals of the oxygen atom. Each bond formed as a result is by one-third an ionic and by two-thirds a covalent bond. The angles and lengths of bonds in a water molecule were precisely determined by optical spectroscopy and X-ray diffraction analysis. An average angle of the *H–O–H* bond is 104.5°, which is slightly different from the value 109.5º for the angle corresponding to the ideal tetrahedral arrangement of the four possible sp^3-orbitals of the oxygen atom. This deviation from the ideal angle results from the tendency of unpaired electrons of the oxygen atoms to repel paired electrons (average interatomic distance of *H–O* is 0.965 Å). This configuration of electrons in a water molecule leads to its electronic asymmetry. The more electronegative oxygen atom tends to attract the electrons of hydrogen atoms, and hydrogen nuclei remain bare. As a result, each of two hydrogen atoms has the partial positive charge σ^+, while the oxygen atom carries the partial negative charge σ localised in the region of uncollectivised orbitals.

Reactions in the liquid phase induced by low-temperature plasma at reduced pressure are described in [24,25]. Different arrangements of electrodes are considered here: anode in the liquid phase; anode and cathode in the gaseous phase (implementing this scheme requires creating the conditions when the resistance between the electrodes is higher than the resistance between the cathode electrode and the treated surface); anode is placed in the gaseous phase and cathode in the solution. The last option is the most promising, as it involves no dissolution of the anode material. The main feature of the systems considered is the appearance of a phase interface between plasma and liquid electrolyte solution, which leads to the emergence of new factors associated with the processes of charge transfer. The space near the solution–gas phase interface has properties similar to those of the cathode space when implementing the processes of the classical glow discharge. They also play the anode role in the liquid phase. Based on this observation, it is concluded that the liquid acts as a bipolar electrode. While charged particles knock out electrons from the surface of a solid electrode, in case of a liquid electrode, charged particles penetrate the solution and interact with it, initiating various chemical reactions, which result in the release of electrons into the gaseous phase. Under these conditions, a limiting factor is not the processes occurring in the gaseous phase, but the concentrations of electrically conductive particles in the liquid phase. Ions induced from plasma into the solution recombine to form chemically active particles or accumulate in the solution, changing its electrical conductivity and other properties [26-28]. The result of this complex physicochemical interaction is obtaining activated solutions. Activated water has a specific composition. The reaction products determining its reactivity are the most detectable. This applies primarily to hydrogen peroxide and superoxide compounds, excited particles and radicals, which play an important role in redox processes [29-31]. It should also be noted that these aqueous solutions after plasma treatment can exhibit some new properties, previously little studied.

The use of plasma-chemically activated aqueous solutions is aimed at increasing the efficiency of disinfection, reducing antimicrobial treatment, and enhancing the toxicological safety, since their composition is rich in peroxides and superoxide compounds [29].

It is achieved by using plasma-chemically activated aqueous solutions, which contain the active substance (hydrogen peroxide), in the processes of disinfection. Hydrogen peroxide is a common classical antiseptic agent. Getting into cells, under action of enzymes (peroxidase and catalase), it breaks down into water and oxygen, which has an antimicrobial effect. After its action, no harmful chemicals remain in the cells, so there is no chemical contamination of food raw materials. Plasma-chemically activated aqueous solutions do not result in unwanted odours and off-tastes and make it unnecessary to use chemical preservatives. Plasma-chemically activated aqueous solutions can be used as a multi-purpose disinfecting, sterilising, and bactericidal agent.

The purpose of this study consists in studying of the effectiveness of treating chicken eggs intended for human consumption with the disinfectants such as the proposed plasma-chemically activated aqueous solutions with various concentrations.

Objectives of the study:

1. Establishing the disinfecting properties of plasma-chemically activated aqueous solutions towards specific pathogenic microflora of eggs.

2. Selecting the modes of plasma-chemical activation of the aqueous solutions to decontaminate the surface of marketable eggs.

3. Studying the changes in the microbiological status of the eggs' surface during storage immediately after their disinfection by plasma-chemically activated aqueous solutions.

Research materials and methods

Water was activated using the special laboratory plasma-chemical unit (Fig. 1) functioning on the basis of the research and production laboratory of the Dnipro State Agrarian and Economic University.

Tap water was activated in plasma discharges of reduced pressure with the voltage 1000–1200 V and current 30.0–200.0 mA, with the subsequent transition, as the electrical conductivity increased, to the mode of non-equilibrium plasma with the following parameters: voltage 400 to 600 V, current up to 150 mA. The content (concentration) of hydrogen peroxide in the activated water was determined by iodometry [30].

The unit operation is described as follows [31]: the input voltage is supplied to step-up transformer. AC voltage from the secondary winding of the transformer is applied to the bridge rectifier, and pulsating DC voltage through the ballast resistor is further supplied to the electrodes of the reactor. Additionally, the reactor anode is connected to the ignition device, which generates pulses of up to 15 kV amplitude and duration of up to 1.5 ms. The pulses are rigidly synchronised with the phase of the pulsating voltage. When the ignition pulse is formed, there is a breakdown of the vacuum space (created by pumping the gaseous phase out of the reactor using the vacuum pump) between the reactor electrodes. As a result of a sharp drop in the resistance, anode current begins flowing, thus creating a discharge. The voltage of the discharge burning is almost constant (750–900 V) and depends on how thin the gas is within the reactor. The magnitude of current of the discharge gap is determined by plasma resistance and by the value of the voltage applied to the system "plasma discharge – ballast regulator." The voltage is regulated under the principle of the phase method, i.e. the average value of the anode voltage supplied to the reactor depends on the phase of pulsating voltage at the anode and the time of the ignition pulse. Plasma appears at the moment of ignition and goes out at the end of the anode voltage ripple. The process repetition frequency is 100 Hz. The discharge current is regulated in the device by changing the ignition moment in relation to the phase of the anode voltage ripple using a synchronising device. In this case, the reactor acts as a power-regulating unit. Plasma discharge parameters are recorded using the devices of the M4200 type, class 4.0. Characteristics of the activated aqueous solutions used as disinfectants in the egg treatment are given in Table 1.

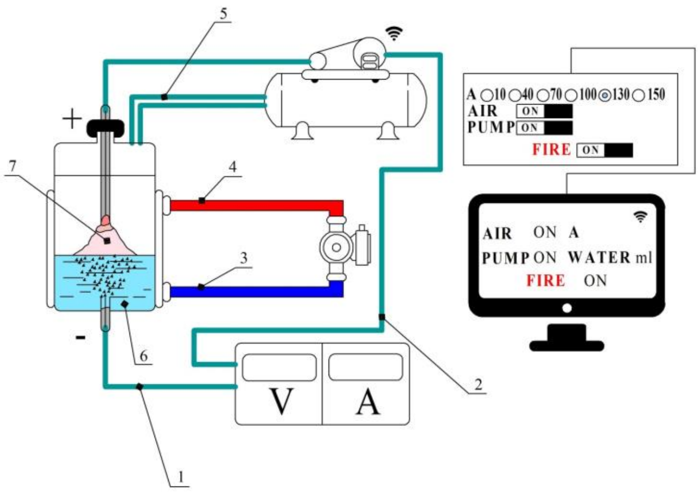

Fig. 1. Laboratory unit for plasma-chemical treatment of water and aqueous solutions:
1, 2 – electrodes and voltage/current measuring devices; 3, 4 – reactor cooling system;
5 – vacuum pump with the vacuum pumping line; 6 – water or aqueous solution; 7 – plasma discharge

Table 1 – Characteristics of water activated by non-equilibrium contact plasma

Expe-riment	Water	Activation time, minutes	Concentration of hydrogen peroxide, mg/l
1 (control)	Tap water	–	–
2	Activated water	3	100
3	Activated water	5	200
4	Activated water	10	300
5	Activated water	20	400
6	Activated water	25	500
7	Activated water	30	600
8	Activated water	60	700

For the tests, the first category chicken eggs (65–74.9 g) produced by the poultry farm *Ptakhofabryka Dnipro* were selected. Accordingly, 8 batches of eggs, 10 pieces in each, were formed. One batch was not subjected to treatment and served as a control. It was kept under conditions absolutely identical to those for the batches of treated eggs. The surface of shells in all other batches was disinfected in the laboratory.

In the course of the studies, the marketable eggs were disinfected by wet treatment with the solutions activated by non-equilibrium contact plasma with the amount of the active substance (hydrogen peroxide) ranging within 100–700 mg/l during 10 minutes. This time of treatment is generally accepted in the egg disinfection technology [9]. Surface wetting was used for visually clean eggs, and the contaminated ones were treated by immersion in plasma-chemically activated solutions. A dirty, unsaleable chicken egg was treated first by immersion for 10 minutes in the test solution, after which the egg surface was washed. The dried egg was further studied bacteriologically. The disinfection quality of the marketable eggs was controlled after 1, 2, 3, 6, 12, and 24 hours. Besides, the marketable eggs were disinfected by washing with solutions activated under the action of non-equilibrium contact plasma with the amount of the active substance (hydrogen peroxide) ranging from 100 to 700 mg/l for 10–60 minutes. The quality of washing the eggs was determined by bacteriological tests after 5, 14, 25, and 30 days, and before washing, the bacterial contamination of the eggs had been established. In addition, a dirty, unsaleable chicken egg was treated by immersing and washing in the activated solution suggested.

By performing the bacteriological quality control of egg disinfection, the presence of viable cells of pathogenic microflora (QMAFAnM, CFU/cm^3, *S. enteritidis*, *E. coli*, *Asp. fumigatus*) was determined on the surface of treated egg shells. After treatment with disinfectant solutions, wipe samples were taken from all eggs by washing with sterile physiological solutions. The samples were inoculated on the nutrient media to detect the pathogenic microflora. For this purpose, standard methods of microbiological analysis were used [32].

Results of the research and their discussion

The technology suggested is based on the objective to improve the quality of eggs by reducing the microbial contamination of the surface, to remove as much salmonella as possible from the eggs, to extend the shelf life of the product, and to choose an all-purpose option for treatment with disinfectant solutions. The problem can be solved by using plasma-chemically activated aqueous solutions as a disinfectant.

Thus, in the course of the studies, the marketable eggs were disinfected for 10 minutes by wet treatment (washing) with solutions activated with non-equilibrium contact plasma where the amount of active substance (hydrogen peroxide) ranged 100–700 mg/l. The results are shown in Table 2.

Analysis of the data in Table 2 shows that the number of bacteria and fungi on the egg shell is significantly reduced due to the use of plasma-chemically activated aqueous solutions as a disinfectant. For example, *E. coli* and *S. Enteritidis* were not detected in the samples at the peroxide concentration as high as 100 mg/l, which is a significant technological result. And at the peroxide concentration 300 mg/l, the total bacterial contamination and the pathogenic microflora under study (*E. coli*, *Asp. fumigatus, S. Enteritidis*) were not found.

It can be explained by the following. One of the possible mechanisms of action of the activated aqueous solutions on the bacteria is the change in the outer layers of a cell, which makes receptors accessible for reactogenic enzymes, for example, lysozyme. Free radicals form a gap in the cell wall, which leads to the loss of selective permeability. Hydrogen peroxide as a part of the activated water causes the destruction of surface structures and internal membranes in microorganisms. The integrity of the cytoplasmic membrane disrupts the work of a number of membrane-related enzymes, such as dehydrogenases, and reduces the effectiveness of the DNA repair systems. The bactericidal activity of hydrogen peroxide and activated water is primarily due to their high oxidative capacity, as well as to the action of toxic products that appear during lipid peroxidation.

Table 2 – Effectiveness of disinfection of the marketable eggs

Exposure, hours	Bacterial and fungal count on the egg shells						
	Total bacterial contamination		*E. coli*		*Asp. fumigatus*		*S. enteritidis*
	Thousand	% of disinfection	Thousand	% of disinfection	Thousand	% of disinfection	Presence
Control sample							
0	380±12.1	–	3.27±0.15	–	1.87±0.07	–	detected
1	385±12.5	–	3.35±0.16	–	1.89±0.08	–	detected
2	390±12.6	–	3.42±0.17	–	1.91±0.09	–	detected
3	392±12.3	–	3.45±0.17	–	1.95±0.09	–	detected
6	396±12.2	–	3.50±0.19	–	1.99±0.10	–	detected
12	398±12.7	–	3.55±0.20	–	2.01±0.11	–	detected
24	401±12.8	–	3.61±0.21	–	2.05±0.12	–	detected
Sample treated with plasma-chemically activated aqueous solutions (100 mg/l of H_2O_2)							
0	0.31±0.04	99.91	0	100.00	0.35±0.01	81.28	not detected
1	0.33±0.05	99.91	0	100.00	0.36±0.02	80.95	not detected
2	0.37±0.07	99.91	0	100.00	0.37±0.02	80.63	not detected
3	0.39±0.09	99.90	0	100.00	0.39±0.03	80.00	not detected
6	0.41±0.10	99.90	0	100.00	0.40±0.04	79.90	not detected
12	0.43±0.11	99.89	0	100.00	0.41±0.04	39.60	not detected
24	0.45±0.12	99.89	0	100.00	0.42±0.05	79.51	not detected
Sample treated with plasma-chemically activated aqueous solutions (300 mg/l of H_2O_2)							
0	0	100.00	0	100.00	0	100.00	not detected
1	0	100.00	0	100.00	0	100.00	not detected
2	0	100.00	0	100.00	0	100.00	not detected
3	0	100.00	0	100.00	0	100.00	not detected
6	0	100.00	0	100.00	0	100.00	not detected
12	0	100.00	0	100.00	0	100.00	not detected
24	0	100.00	0	100.00	0	100.00	not detected

Peroxidation affects the ribosome proteins, causing their destruction. Superoxide compounds formed also contribute to the destruction of membrane structures. The action of hydrogen peroxide or activated water causes the local destruction of the integral cell wall and disruption of bacterial cell permeability in the first minutes of contact. The result is the possibility of sterilising the egg surface, as well as removing salmonella from it.

The effectiveness of dipping eggs in the 6% hydrogen peroxide solution to eliminate *Salmonella typhimurium* from membranes of the egg shell was assessed in [33]. The absorption of water from hatching eggs of broilers immersed in tap water was studied. Repeated dipping increased water absorption by 86%, compared with single-time immersion. Double immersion of eggs contaminated by *S. typhimurium* in a 6% hydrogen peroxide solution reduced the average number of organisms in the eggshells by 95%, and the number of *S. typhimurium*-positive eggs by 55%, compared with the contaminated group not subjected to treatment. Immersion of eggs in the 6% hydrogen peroxide solution did not affect the hatchability adversely. Consequently, effectiveness of aqueous solutions of synthetic hydrogen peroxide for decontamination of these pathogens was confirmed at the first stages of the studies.

In the course of our work, bacteriological studies of marketable eggs during storage were carried out. The marketable eggs were disinfected by wetting with solutions activated by non-equilibrium contact plasma with the active substance (hydrogen peroxide) ranging 100–700 mg/l for 10 minutes. The bacteriological studies were conducted for one month in order to establish the possible development of pathogenic microflora on the surface of the marketable eggs (Table 3).

The results given in Table 3 allow us to state that plasma-chemically activated aqueous solutions with the peroxide concentration 300 mg/l can completely destroy pathogenic microflora, as evidenced by the total absence of QMAFAnM, CFU/cm^3, *S. enteritidis*, *E. coli*, *Asp. fumigatus* in the samples, and provide a long-lasting disinfection effect, because no pathogenic microflora appeared on the surface of the eggs during one month. It will allow extending the shelf life of marketable eggs and protecting consumers from the impact of pathogenic microflora. The above data on the effectiveness of egg decontamination are somewhat similar to the findings of [34-36]. However, the difference in the results can be significant, when we compare the studied process of obtaining plasma-chemically treated water and the combined use of slightly acidic electrolysed water and ultraviolet exposure to improve the internal quality of eggs during storage.

This effect can be explained by the following. One of the main differences of plasma-chemical solutions from solutions of chlorine, sodium hypochlorite, hydrogen peroxide, chlorine dioxide is that in the former, antagonist substances can exist for a long time, whereas in simple solutions, they are involved in reactions of neutralisation and mutual destruction, and besides, they quickly disappear in highly mineralised solutions. That is, the solution, when entering the pores of a shell, can maintain the relative sterility of a chicken egg for a long time.

Besides, bacteriological studies of unsaleable eggs have proved useful too. Contrary to the popular belief, bacteria on an eggshell cannot be destroyed with soapy water, but baking or calcined soda will help. The main difference of calcined soda is the stronger alkaline reaction (pH=11 v pH=8). An alkaline medium is harmful to microorganisms, and its action is similar to that of a UV-lamp. However, it takes some time to achieve the result. In the solution of baking soda, an egg is disinfected for 30 minutes, and for calcined soda, the time is 10 minutes [9].

Findings of the study show that plasma-chemically treated water has the alkaline medium of more than 10. This factor has a positive effect on decontamination of pathogenic microorganisms. As a consequence, disinfection of egg surfaces will be more effective.

The quantity of contaminated eggs affected by pathogenic microflora and the dynamics of changes in the contamination under action of the disinfectant suggested are shown in Table 4.

Analysis of the data from Table 4 have shown that washing of the shell even in dirty eggs with the use of plasma-chemically activated water with increased concentration of peroxides allows disinfecting the surface completely. The peroxide concentration 400 mg/l [37,38] in plasma-chemically activated aqueous solutions makes it possible to get rid of coliform bacteria, staphylococci, salmonella, and spore-forming microflora even when treating dirty chicken eggs, and thus to sell them. Besides, the outer appearance of eggs significantly improves [39]. This testifies to a wide range of applications of the suggested technology of egg surface disinfection.

The use of plasma-chemically activated aqueous solutions reduced the contamination of eggs with bacteria and fungi. At the peroxide concentration 300 mg/l, the eggs were sterile, and retained their sterility for 30 days. This can significantly increase their shelf life and protect consumers from pathogenic microorganisms.

Table 3 – Bacteriological studies of marketable eggs during storage

Exposure, days	**Microbiological parameters**	**Control**	**Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l**						
			100	**200**	**300**	**400**	**500**	**600**	**700**
0	QMAFAnM, CFU/cm^3	$3.9\cdot10^6$	$3.0\cdot10^6$	<10	0	0	0	0	0
	S. enteritidis	D	ND	ND	ND	ND	ND	ND	ND
	E. coli	D	ND	ND	ND	ND	ND	ND	ND
	Asp. fumigatus	D	D	ND	ND	ND	ND	ND	ND
5	QMAFAnM, CFU/cm^3	$4.5\cdot10^6$	$3.1\cdot10^6$	<10	0	0	0	0	0
	S. enteritidis	D	ND	ND	ND	ND	ND	ND	ND
	E. coli	D	ND	ND	ND	ND	ND	ND	ND
	Asp. fumigatus	D	D	ND	ND	ND	ND	ND	ND
14	QMAFAnM, CFU/cm^3	$6.7\cdot10^6$	$3.2\cdot10^6$	<10	0	0	0	0	0
	S. enteritidis	D	ND	ND	ND	ND	ND	ND	ND
	E. coli	D	ND	ND	ND	ND	ND	ND	ND
	Asp. fumigatus	D	D	ND	ND	ND	ND	ND	ND
25	QMAFAnM, CFU/cm^3	$7.8\cdot10^6$	$3.3\cdot10^6$	<10	0	0	0	0	0
	S. enteritidis	D	ND	ND	ND	ND	ND	ND	ND
	E. coli	D	ND	ND	ND	ND	ND	ND	ND
	Asp. fumigatus	D	D	ND	ND	ND	ND	ND	ND
30	QMAFAnM, CFU/cm^3	$11.7\cdot10^6$	$3.5\cdot10^6$	<10	0	0	0	0	0
	S. enteritidis	D	ND	ND	ND	ND	ND	ND	ND
	E. coli	D	ND	ND	ND	ND	ND	ND	ND
	Asp. fumigatus	D	D	ND	ND	ND	ND	ND	ND

Note: D – detected; ND – not detected.

Table 4 – Bacteriological studies of unsaleable contaminated chicken eggs treated with plasma-chemically activated solutions, number of eggs

Microbiological parameters	**Control**	**Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l**						
		100	200	300	400	500	600	700
Coliform bacteria	10	8	3	1	–	–	–	–
Staphylococci	6	4	3	2	1	–	–	–
Salmonella	8	6	3	1	–	–	–	–
Spore-forming bacteria	9	7	5	3	2	–	–	–

Plasma-chemically activated aqueous solutions have a specific composition, including hydrogen peroxide and superoxide compounds, excited particles and radicals, which play an important role in redox processes. Hydrogen peroxide is an antiseptic agent: getting into cells, under action of enzymes, it breaks down into water and oxygen, producing an antimicrobial effect and leaving no harmful chemicals in the cells. We should note that these aqueous solutions after plasma treatment may exhibit new properties, little studied previously. Activating aqueous solutions causes a number of specific physical and chemical effects, which can become a starting point for new advanced technologies [40,41].

The surface of the eggs has retained the desired appearance: the eggshell is clean, with a whitening effect and no unpleasant odour. The eggs do not contain peroxides and other toxic chemical compounds, which confirms the chemical purity and safety of the disinfecting agent, as well as the possibility to obtain raw materials that meet the European standards and requirements to food products and are competitive.

Plasma-chemically activated aqueous solutions can fully replace antiseptic agents and thus maintain the chemical purity of the resulting product. These solutions will be able to replace the classic chemical antiseptics and at the same time be safe, without any chemical compounds undesirable in the human diet.

The technological solution proposed is a method of disinfection, which can be used to obtain eggs with the reliably treated surface and longer shelf life, as compared with those disinfected with the traditional methods. These eggs are completely safe for consumers and can be stored with no difficulties and no extra costs.

Plasma-chemically activated aqueous solutions gently remove the dirt from the surface of eggs without damaging the protective shells. During the long contact of eggs with plasma-chemically activated solutions with high concentration of peroxides, the solution can enter the pores of a shell, thus disinfecting them and destroying pathogenic microflora. The solutions suggested, due to their specific chemical composition, can preserve their disinfectant properties for a long time. Once having entered an egg pore, they can resist the pathogenic microflora that tries to penetrate there. Such properties will be maintained until the solution in the egg pore completely dries out.

The activated aqueous solutions obtained, as compared with the existing disinfectants, feature high antimicrobial and sporocidal activity, low cost, high efficiency, and low toxicity. This agent has no irritating properties, which greatly simplifies the work with it. These solutions can be used to sanitise equipment, inventory, containers, production surfaces, and warehouses at poultry farms and poultry processing plants. The shelf life of the working plasma-chemically activated aqueous solution is 6 months from the date of its manufacture, provided it is stored in a closed container [42].

Plasma-chemically activated aqueous solutions were prepared on the basis of the specialised laboratory for plasma treatment of technological solutions of food processors. All studies were performed in the Research and Production Laboratory of the Department of Technology of Storage and Processing of Agricultural Products of the Dnipro State Agrarian and Economic University.

Conclusion

Analysis of the possibilities of using plasma-chemically activated aqueous solutions directly as disinfectants of a chicken egg surface has shown the prospects of this technological method. The use of electrochemical activation can facilitate the process and reduce the cost of obtaining the products in many cases, taking into account the cost of energy and the time spent on activation. Below, the results of the study are listed.

1. The disinfecting properties of plasma-chemically activated aqueous solutions towards the pathogenic microflora of eggs have been established, as the research data confirm the complete destruction of pathogenic microflora on the surface of marketable eggs.
2. The modes of plasma-chemical activation of aqueous solutions in order to disinfect the marketable eggs have been selected, namely, the optimal parameter for decontaminating the egg surface was the solutions' peroxide concentration 300 mg/l and more. It is proved by almost complete sterility of the eggshell. The treatment mode with the peroxide concentration 400 mg/l allowed disinfecting even unsaleable, dirty eggs and eggs heavily contaminated with pathogenic microorganisms.
3. The study of the shelf life of disinfected eggs shows that marketable eggs disinfected with plasma-chemically activated aqueous solutions retain the relative sterility of the shell for 30 days, which is an important technological result.

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ДЕЗІНФЕКЦІЯ ТОВАРНИХ ЯЄЦЬ ПЛАЗМОХІМІЧНО АКТИВОВАНИМИ ВОДНИМИ РОЗЧИНАМИ

О. А. Півоваров, доктор техн.наук, професор, *E-mail* apivo@ua.fm
О. С. Ковальова, кандидат техн. наук, доцент, *E-mail*: livre@i.ua
В.С. Кошулько, кандидат техн. наук, доцент, *E-mail*: vitaliykoshulko@gmail.com
Кафедра технології зберігання і переробки сільськогосподарської продукції,
Дніпровський державний аграрно-економічний університет, вул. Сергія Єфремова, 25, м. Дніпро, Україна, 49600

Анотація. Одним із найважливіших факторів безпеки продуктів харчування є мікробіологічна чистота, як сировини, так і готового продукту. Допустимий рівень мікробіологічної забрудненості досягається комплексом санітарно-гігієнічних прийомів при виробництві, реалізації і споживанні харчових продуктів. Запропоновано проводити знезараження яєць шляхом їх обробки плазмохімічно активованими водними розчинами, з метою зниження мікробної забрудненості поверхні яєць і деконтамінації їх від сальмонел. Поверхня яєць знезаражується водними розчинами активованими під дією контактної нерівноважної плазми з різним часом активації таких розчинів. Плазмохімічно активовані водні розчини виконують функцію високоякісного дезінфектанту, мають в своєму складі діючу речовину – пероксид водню концентрацією від 100 до 700 мг/л і володіють вираженими антисептичними властивостями. Дослідження показали, що кількість бактерій і грибів на шкарлупі яйця значно знизилась при використання в якості дезінфектора плазмохімічна активованих водних розчинів. Так *E. coli* і *S. Enteritidis* були відсутні в зразках вже при концентрації пероксидів 100 мг/л. А при концентрації пероксидів 300 мг/л повністю відсутня загальне бактеріальне забруднення і досліджувана патогенна мікрофлора (*E. coli, Asp. fumigatus, S. Enteritidis*). Крім того в ході роботи були проведені бактеріологічні дослідження товарних яєць при зберіганні. Так плазмохімічно активовані водні розчини з концентрацією пероксидів 300 мг/л не тільки здатні повністю знищити патогенну мікрофлору, про що свідчить повна відсутність в досліджуваних зразках КМАФАнМ, КУО/см^3, *S. enteritidis, E. coli, Asp. fumigatus*, а й тривалий ефект знезараження, оскільки протягом місяця на поверхні яєць не з'явилась патогенна мікрофлора. Це дозволить подовжити строки зберігання товарних яєць. Дослідження мікробіологічних показників шкарлупи брудних яєць, які оброблялись плазмохімічно активованими водними розчинами показали, що концентрація пероксидів 400 мг/л дає можливість позбавитись від бактерій групи кишкової палички, стафілококів, сальмонел, спороутворюючої мікрофлори, що дозволить їх реалізовувати. Покращується зовнішній вигляд сировини, що говорить про широкий спектр застосування запропонованої технології знезараження поверхні яєць.

Ключові слова: плазмохімічна активація, водні розчини, дезінфектант, пероксид водню, курячі яйця, контамінація.